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Hormonal regulation of postnatal chicken preadipocyte differentiation in vitro☆

T.G. Ramsay*, R.W. Rosebrough

Growth Biology Laboratory, USDA-ARS, Beltsville, MD 20705, USA

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Abstract

This study was designed to develop a culture system from the stromal-vascular fraction of chicken adipose tissue that can be used to characterize hormones that promote preadipocyte differentiation. Abdominal adipose tissue was excised from 2 to 4-week-old male broilers (*Gallus domesticus*) by sterile dissection. The stromal-vascular cell fraction from the adipose tissue was isolated by collagenase digestion, filtration, and subsequent centrifugation. These preadipocytes were seeded in six well culture plates and proliferated to confluency in 10% fetal bovine serum in DMEM/F12 (50:50) medium. At confluency, experiments were initiated to determine hormonal requirements for differentiation. Insulin (100 nM) stimulated expression of citrate lyase and *sn*-glycerol-3-phosphate dehydrogenase relative to lactate dehydrogenase in the presence of 2.5% chicken serum ($P<0.05$), but not with 10% chicken serum ($P>0.05$). Triiodothyronine (T_3, 1 nM) and insulin-like growth factor 1 (100 ng/ml) had no effect on differentiation. Dexamethasone (Dex, 1 μM) stimulated differentiation in 2.5 or 10% chicken serum ($P<0.05$). Insulin, Dex and 2.5% chicken serum stimulated enzymatic differentiation to the extent of 10% chicken serum, but heparin (10 U/ml) addition, in combination with insulin and Dex was necessary to stimulate lipid filling of adipocytes.

Keywords: Preadipocyte; Differentiation; Cell culture

1. Introduction

Relatively few studies have attempted to culture preadipocytes from chickens. These studies have primarily focused on identifying factors regulating preadipocyte replication (Butterwith, 1997). The rapid postnatal growth of avian adipose tissue and enlargement of the abdominal fat pad suggest that differentiation and lipid filling are also important factors in controlling fat pad size (Leclercq, 1984). Development of methods to assess the differentiation process and the metabolic maturation of the adipocyte may provide clues to regulate this metabolism. Whereas adipose tissue is the primary lipid-synthesizing organ in the mammal, the liver serves this function in the bird while the adipose takes up the preformed lipid.

The present study was designed to develop a method to chronically culture the stromal-vascular fraction of chicken adipose tissue and to optimize conditions for fat cell formation. Previous studies have utilized high serum concentrations (10–20%

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*Corresponding author. Growth Biology Laboratory, USDA-ARS, BARC-East, Building 200, Room 201, Beltsville, MD 20705, USA. Tel.: +1-301-504-5958; fax: +1-301-504-8623.
E-mail address: tramsay@anri.barc.usda.gov (T.G. Ramsay).

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chicken serum) in the medium to induce differentiation (Cryer et al., 1987; Butterwith and Griffin, 1989; Wu et al., 2000). This high serum concentration does not permit an accurate assessment of the role of individual hormones in differentiation, due to interference from the myriad of hormones present in serum. The present study was designed to identify hormones or peptides that can promote adipocyte formation with low serum concentrations in the culture medium. This study was also designed to develop a culture system and medium that permits adipocyte differentiation without requiring high concentrations of chicken serum.

2. Materials and methods

2.1. Cell culture

Male Shaver broilers were used as a source of adipose tissue. Birds were grown under standard management conditions from 1 to 28 days of age. Birds were fed a common diet (21% crude protein) ad libitum. Abdominal adipose tissue was excised from 2-to 4-week-old birds by sterile dissection following rapid decapitation. Care, handling and all procedures performed with birds were approved by the Beltsville Area Research Center Animal Care and Use Committee.

Adipose tissue (1 g) was minced into sections of approximately 1 mm^2 with scissors and incubated with 5 ml of digestion buffer (Dulbecco's modified Eagle's Medium/Ham's F12 medium (50:50) (DMEM/F12), 100 mM *N*-2 hydroxyethylpiperazine-*N*′-2 ethanesulfonic acid (HEPES), 1.5% bovine serum albumin, pH 7.4) supplemented with 2 mg/ml collagenase (Type 1, Worthington Biochemical Co., Lakewood, NJ). A fivefold excess of buffer (37 °C) was added to the digestion flask after 30 min of incubation at 37 °C in a shaking water bath (90 oscillations/min). Flask contents were mixed and filtered through nylon screens with 250 and 20-μm mesh openings to remove undigested tissue and large cell aggregates. The filtered cells were centrifuged at $500\times g$ for 7 min to separate the floating adipocytes from the pellet of stromal-vascular cells.

The stromal-vascular cells were then incubated with erythrocyte lysis buffer (0.154 M NH_4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA) at room temperature (Hauner et al., 1989) for 10 min, followed by centrifugation at $500\times g$ for 10 min. The stromal-vascular cell pellet was washed with DMEM/F12, centrifuged and resuspended in plating medium (10% fetal bovine serum, DMEM/F12, 1×10^5 U/l penicillin G, 100 mg/l streptomycin, 25 mg/l amphotericin B, 2×10^5 U/l mycostatin).

Aliquots of the stromal-vascular fraction were removed, stained with Rappaport's stain and counted on a hemocytometer. Stromal-vascular cells (including the preadipocytes) were seeded on six well plates at a density of 5×10^4 cells/well in plating medium. Cells were maintained at 37 °C in a humidified, 5% CO_2 atmosphere. Cells were maintained in plating medium until confluency (d 3–4), when differentiation was initiated and experiments were begun.

2.2. Experimental design

This study was performed to develop a culture system and medium that permits adipocyte differentiation without requiring high concentrations of chicken serum (10–20%). Thus, a series of experiments were performed to find alternatives. Each experiment was repeated four times with isolated cell cultures derived from four different animals.

2.2.1. Use of phosphodiesterase inhibitors to promote differentiation

The first experiment introduced the combination of dexamethasone (1 μM, Sigma Chemical Co., St. Louis, MO) and 10 mM isobutylmethylxanthine (IBMX, Sigma Chemical Co.) at confluency for a period of 48 h, and then replaced with either 2.5 or 10% chicken serum (Sigma Chemical Co.) supplemented with or without 100 nM porcine pancreatic insulin (#I-5523 Sigma Chemical Co.). This regimen of hormonal treatments has been successful for the induction of adipocyte formation in cultures derived from pig adipose tissue (Ramsay, 2000), rat adipose tissue (Bjorntorp et al., 1978) and the mouse cell line 3T3-L1 (Wise et al., 1984).

2.2.2. Introduction of individual hormones into low vs. high serum containing medium

Several hormones, demonstrated to induce differentiation in cultures derived from other species, were tested in combination with either 2.5 or 10% chicken serum. These hormones included insulin (100 nM), dexamethasone (1 μM), recombinant human insulin-like growth factor I (IGF-I, 100 ng/ml, Sigma Chemical Co.) and triiodothyronine (T_3, 1 nM, Sigma Chemical Co.). These are

pharmacological concentrations that have been previously used in primary cultures derived from adipose tissues of other species (Ramsay et al., 1989a,b,c). Hormones were not evaluated that have previously been demonstrated to be ineffective in promoting chicken preadipocyte differentiation (Butterwith, 1997). Test hormones and chicken serum were introduced at confluency with media changes every 2 days until harvest at day 14 of culture.

2.2.3. Supplementation of hormone combinations in low vs. high serum containing medium

Hormone combinations were tested to evaluate potential interactions that could augment adipocyte formation. For example, the combination of insulin and corticosteroid have synergistic effects on porcine preadipocyte differentiation (Ramsay et al., 1989b; Suryawan et al., 1997) while T_3 and insulin have additive effects on differentiation of Ob17 preadipocytes (Gharbi-Chihi et al., 1984). Thus, the hormone combination of insulin (100 nM) and dexamethasone (1 μM), and the combination of insulin (100 nM) and T_3 (1 nM) were tested on post-confluent chicken preadipocytes. Hormone combinations were supplemented to 2.5 or 10% chicken serum and introduced at confluency with media changes every 2 days until harvest at day 14 of culture.

2.3. Enzyme analyses

Cell cultures in six well plates were washed with serum free DMEM/F12 following hormone treatment and then 1.0 ml of an ice-cold homogenizing buffer was added. Homogenizing buffer for the analysis of *sn*-glycerol-3-phosphate dehydrogenase (GPDH), citrate lyase (CL), and lactate dehydrogenase (LDH) was as described by Ramsay et al. (1987). Cells were scraped free of the culture plates and collected in microfuge tubes prior to sonication for 1 min at the maximum setting for a microtip horn attached to a Fisher sonicator. Homogenates were centrifuged at $14\,000 \times g$ for 5 min at 4 °C in microfuge tubes. The supernatants were used for analysis. *Sn*-glycerol-3-phosphate dehydrogenase (EC 1.1.1.8) activity was determined by the procedure of Wise and Green (1978). Citrate lyase (EC 4.1.3.8) activity was assessed by the procedures of Cottam and Srere (1969). These enzymes have been demonstrated to be associated with the differentiation of preadipocytes (Cryer, 1985). Lactate dehydrogenase (EC 1.1.1.27) was assayed according to Neilands (1955) and served as an internal control as its activity is not associated with the process of preadipocyte differentiation. Assays were linear for sample concentration and time. Variations between duplicate determinations never exceeded 5%. Protein concentrations of cell culture homogenates were determined by the method of Bradford (1976), except for experiments including a T_3 treatment, for which a modified Lowry procedure was used (Nerurkar et al., 1981). Data were calculated as nanomoles NAD(H) or NADP(H) utilized/min/mg protein.

For some experiments, wells from tissue culture plate were stained for histochemical and morphological analysis on day 14 of culture. Cells were stained for lipid with oil Red O (Hausman, 1981). The number and size of adipocyte clusters were quantified for 10 microscopic fields at 100× magnification.

2.4. Statistical analysis

Animal to animal variation is always a significant problem in the analysis of data from primary cultures. Consequently, the data are expressed on a relative percentage basis to correct for the variation among animals (trial). The activity of cultures exposed to the basal medium for each trial in each experiment was given a relative value of 100 and the activity of cultures exposed to treatment media for each trial in each experiment was calculated as a percentage of this value.

Data were subjected to analysis of variance. Where significant effects were found, the detection of mean separation was computed by Student–Newman–Keuls test (SIGMASTAT version 1.03, SPSS Science, Chicago, IL). Significant differences were defined at the 95% confidence level, unless otherwise indicated. Data are expressed as the mean ± S.E.

3. Results

Supplementation of medium containing 2.5% chicken serum with 10 mM IBMX and 1 μM dexamethasone to induce differentiation, followed by insulin treatment to promote lipid filling, had no greater effect than 2.5% chicken serum without additional hormone supplementation on enzyme activities associated with preadipocyte differentia-

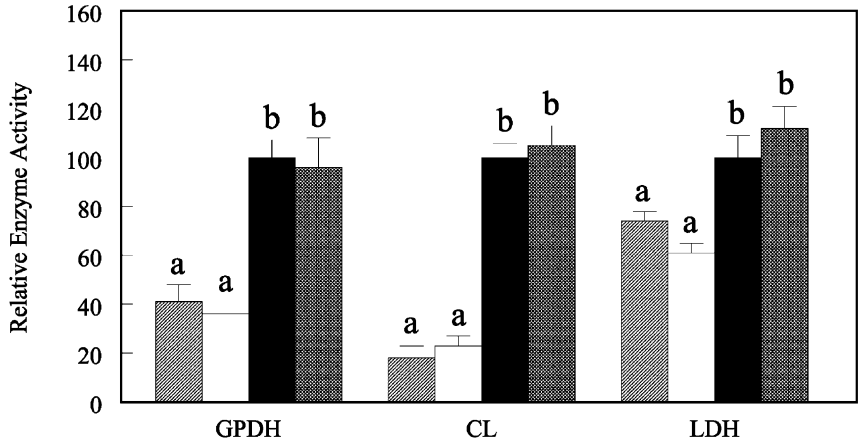

Fig. 1. Relative enzyme activity in response to incubation with: 2.5% chicken serum for 9 days post confluency (hatched bar); 2.5% serum, 10 mM IBMX and 1 μM dexamethasone for 48 h, followed by 7–8 days of incubation with 2.5% chicken serum and 100 nM insulin (open bar); 10% chicken serum for 9 days post confluency (black bar); or 10% chicken serum, 10 mM IBMX and 1 μM dexamethasone for 48 h, followed by 7–8 days of incubation with 10% chicken serum and 100 nM insulin (gray hatched bar). Mean ± S.E. for four trials; values are expressed as percent of activity of cultures exposed to 10% chicken serum per μg protein. Means not sharing a common superscript are significantly different ($P<0.05$, $n=4$). GPDH, *sn*-glycerol-3-phosphate dehydrogenase; CL, citrate lyase; LDH, lactate dehydrogenase.

tion (Fig. 1, $P>0.05$). Similarly, this treatment regimen supplemented to 10% chicken serum was no more effective than 10% chicken serum alone at promoting differentiation as assessed by relative expression of differentiation associated enzyme activities ($P>0.05$). Chicken serum at 2.5% induced relatively less expression of GPDH and CL than 10% serum ($P<0.05$).

Supplementation of 100 nM insulin to 2.5% chicken serum was effective in promoting an 58% increase in GPDH activity, a 102% increase in CL activity ($P<0.05$, Fig. 2a) but no effect on LDH activity ($P>0.05$), indicative of differentiation. However, the activity was still less than in cultures exposed to 10% chicken serum. Insulin supplementation to 10% chicken serum did not alter enzyme activities ($P>0.05$).

Triiodothyronine (1 nM) stimulated a 60–63% increase in LDH activity ($P<0.05$), but had no effect on GPDH or CL in either 2.5 or 10% chicken serum (Fig. 2b). IGF-I supplementation (100 ng/ml) to culture medium had no effect on the activity of any of the monitored enzymes (Fig. 2c).

Dexamethasone (1 μM) induced a 54% increase in GPDH activity ($P<0.05$) and a 91% increase in CL activity ($P<0.05$) when supplemented to 2.5% chicken serum (Fig. 2d). Supplementation of dexamethasone to 10% chicken serum produced approximately a 30% increase in the activity of both enzymes ($P<0.05$).

The combination of 100 nM insulin and 1 nM T_3 produced a 48% increase in GPDH activity ($P<0.05$) and an 87% increase in CL activity ($P<0.05$) in cultures exposed to 2.5% chicken serum (Fig. 3a). No effect was detected for this hormone combination on CL and GPDH in cultures incubated in 10% serum. LDH activity was elevated by approximately 50% because of this hormonal milieu in cultures treated with either 2.5 or 10% serum ($P<0.05$).

Dexamethasone (1 μM) in combination with 100 nM insulin stimulated GPDH and CL activity in cultures exposed to 2.5% chicken serum (Fig. 3b). This combination of hormones induced approximately a twofold increase in GPDH and CL activities, relative to 2.5% serum ($P<0.05$). This hormonal combination produced a medium that was equivalent to 10% chicken serum for its capacity to induce differentiation. A much smaller response was observed when this combination of hormones was supplemented to 10% chicken serum. Approximately a 40% increase in CL and GPDH activity was measured in cultures treated with 10% chicken serum ($P<0.05$). LDH activity was not affected by the combination of insulin and dexamethasone ($P>0.05$).

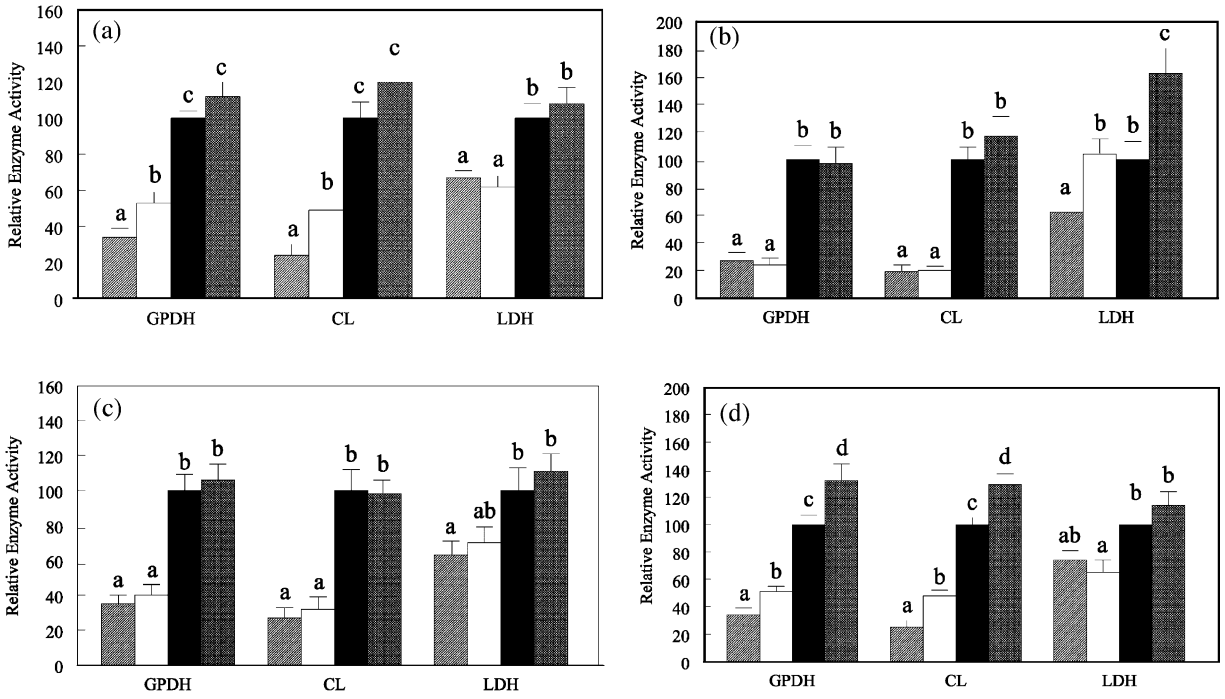

Fig. 2. (a) Relative enzyme activity in response to incubation with: 2.5% chicken serum (hatched bar); 2.5% serum + 100 nM insulin (open bar); 10% chicken serum (black bar); or 10% chicken serum + 100 nM insulin (gray bar). (b) Relative enzyme activity in response to incubation with: 2.5% chicken serum (hatched bar); 2.5% serum + 1 nM triiodothyronine (open bar); 10% chicken serum (black bar); or 10% chicken serum + 1 nM triiodothyronine (gray bar). (c) Relative enzyme activity in response to incubation with: 2.5% chicken serum (hatched bar); 2.5% serum + 100 ng/ml IGF-I (open bar); 10% chicken serum (black bar); or 10% chicken serum + 100 ng/ml IGF-I (gray bar). (d) Relative enzyme activity in response to incubation with: 2.5% chicken serum (hatched bar); 2.5% serum + 1 μM dexamethasone (open bar); 10% chicken serum (black bar); or 10% chicken serum + 1 μM dexamethasone (gray bar). Cultures were maintained on treatment media from confluency (days 3–4) until harvest (day 14). Mean ± S.E. for four trials; values are expressed as percent of activity of cultures exposed to 10% chicken serum per μg protein. Means not sharing a common superscript are significantly different ($P < 0.05$, $n = 4$). GPDH, *sn*-glycerol-3-phosphate dehydrogenase; CL, citrate lyase; LDH, lactate dehydrogenase.

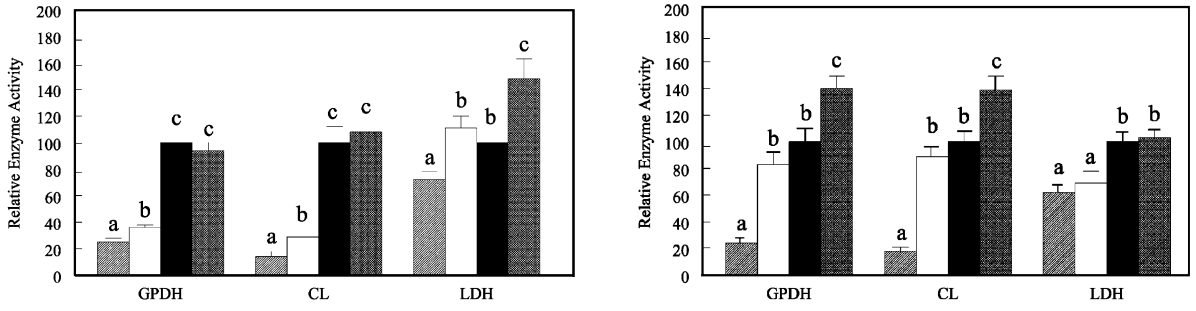

Fig. 3. (a) Relative enzyme activity in response to incubation with: 2.5% chicken serum (hatched bar); 2.5% serum + 100 nM insulin + 1 nM T_3 (open bar); 10% chicken serum (black bar); or 10% chicken serum + 100 nM insulin + 1 nM T_3 (gray bar). (b) Relative enzyme activity in response to incubation with: 2.5% chicken serum (hatched bar); 2.5% serum + 100 nM insulin + 1 μM dexamethasone (open bar); 10% chicken serum (black bar); or 10% chicken serum + 100 nM insulin + 1 μM dexamethasone (gray bar). Cultures were maintained on treatment media from confluency (days 3–4) until harvest (day 14). Mean ± S.E. for four trials; values are expressed as percent of activity of cultures exposed to 10% chicken serum per μg protein. Means not sharing a common superscript are significantly different ($P < 0.05$, $n = 4$). GPDH, *sn*-glycerol-3-phosphate dehydrogenase; CL, citrate lyase; LDH, lactate dehydrogenase.

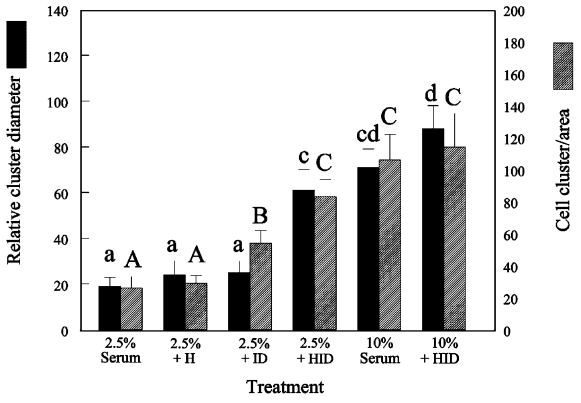

Fig. 4. Morphological analysis of adipocytes in vitro. Cultures were stained for lipid with oil Red O on day 14 of culture after 9 days exposure to treatment media (Hausman, 1981). Treatment media included: 2.5% chicken serum; 2.5% chicken serum + 100 nM insulin + 1 μM dexamethasone (ID); 2.5% chicken serum + 10 U heparin/ml medium (H); 2.5% chicken serum + 10 U heparin/ml medium + 100 nM insulin + 1 μM dexamethasone (HID); 10% chicken serum; or 10% chicken serum + 10 U heparin/ml medium + 100 nM insulin + 1 μM dexamethasone (HID). Mean ± S.E. for three trials. The size (left *y* axis, black bars) and number (right *y* axis, hatched bars) of adipocyte clusters were quantified for 10 microscopic fields at 100× magnification. Means not sharing a common superscript letter are significantly different ($P < 0.05$, $n = 3$ cultures).

These cells still did not accumulate significant quantities of lipid (Fig. 4). Heparin (10 U/ml medium; Montalto and Bensadoun, 1993) was added to medium to stimulate lipoprotein lipase activity and thus promote lipid filling. Histochemical quantification of fat cell cluster size and diameter demonstrated that addition of heparin to the hormonal combination of insulin and dexamethasone increases the lipid accumulation by avian adipocytes by threefold ($P < 0.05$). Heparin alone had no effect on adipocyte number when supplemented to 2.5% chicken serum ($P > 0.05$). Insulin plus dexamethasone stimulated approximately a 107% increase in adipocyte cluster number relative to 2.5% chicken serum ($P < 0.05$), but the hormone combination had no effect on cluster size ($P > 0.05$). Neither heparin addition nor the combination of insulin and dexamethasone had any effect on lipid accumulation in the presence of 10% serum (data not shown). The combination of 2.5% chicken serum, 1 μM dexamethasone, 100 nM insulin and 10 U heparin/ml produced adipocytes comparable to 10% chicken serum based upon this histochemical analysis, with approximately 35–40% of the cells differentiating into lipid accumulating adipocytes.

4. Discussion

The regulation of preadipocyte differentiation has not been well characterized in the chicken. A major contribution to this lack of understanding has been the inability to isolate the actions of an individual hormone on the preadipocyte. This inability to segregate specific hormone effects is the result of the use of high serum concentrations, which can dilute the specific hormone or supply counterregulatory hormones. The present study was designed to develop a method to use low serum concentrations in vitro for stimulating adipocyte differentiation, thus permitting identification of important regulatory hormones for differentiation. The eventual goal would be to develop serum free culture conditions to evaluate specific hormones and growth factors for their effects on differentiation.

The present study did not evaluate the potential actions of hormones or growth factors on preadipocyte proliferation. Other studies have carefully evaluated the mitogenic properties of hormones on chicken preadipocytes (for review, please see Butterwith, 1997). In the present study, the experimental design tested hormones and growth factors once the cells were confluent and at a relative growth arrest due to contact inhibition. This would limit proliferative effects of hormones and growth factors to some extent. However, we would presume that some post-confluent mitosis occurs in response to mitogenic hormones, as in mammalian adipose tissue (Cornelius et al., 1994).

One of the more common methods to induce preadipocyte differentiation is through increasing cellular cAMP concentrations, thus affecting tyrosine kinase activity and cellular signaling for differentiation induction (Bjorntorp et al., 1978). Increasing intracellular cAMP has been used in combination with glucocorticoids to stimulate differentiation of preadipocytes from a variety of mammalian species, followed by insulin treatment to promote lipid accretion (Ailhaud, 1982; Bjorntorp et al., 1978; Ramsay, 2000). The present study demonstrated that this regimen was ineffective for differentiating the chicken preadipocyte. One contributor to this ineffectiveness could be inhibition of lipoprotein lipase synthesis by cAMP (Bensadoun and Marita, 1986), which would inhibit any

lipid accretion by the cells as they differentiate. However, IBMX was incubated with the cells for only 48 h and most likely would not have long term effects on LPL synthesis. More importantly, any changes in cAMP level that were induced by IBMX did not result in any changes the activity of the enzyme markers for differentiation. These data indicate that the avian preadipocyte uses different intracellular signaling mechanisms for regulation of differentiation than the mammalian preadipocyte.

Insulin is considered an adipogenic hormone due to its lipogenic actions and its role in the partitioning of energy to fat accretion. Cryer et al. (1987) were the first to examine the potential adipogenic actions of insulin on chicken preadipocytes. No detectable adipogenic activity was attributable to insulin in that culture system. The lack of insulin effect may have been due to the use of very high (20%) serum concentrations in the cultures. The present study demonstrates that porcine insulin stimulates an increase in the activity of known enzyme markers for preadipocyte differentiation, if low concentrations of serum (2.5%) are used. Porcine insulin has been shown to bind to the chicken insulin receptor and stimulate receptor tyrosine kinase activity (Simon et al., 1986). The stimulatory effects of porcine insulin were limited but significant, although use of chicken insulin might produce an even greater response. The effects of porcine insulin are masked by use of high concentrations of serum (10%), as shown in the present study.

Triiodothyronine is an essential inducer of differentiation for the mouse Ob17 preadipocyte cell line (Gharbi-Chihi et al., 1984) and can stimulate porcine preadipocyte differentiation in serum free cultures (Hausman, 1989) or serum supplemented cultures (Ramsay et al., 1989c). However, the present study could not demonstrate an effect of 1 nM T_3 on avian preadipocyte differentiation. Triiodothyronine has systemic inhibitory effects on adipose accretion in birds, perhaps through its actions on the liver (Rosebrough et al., 1992) and not preadipocyte differentiation. Insulin was still able to promote differentiation when used in combination with T_3 (Fig. 3a) to a similar extent as when insulin was used alone (Fig. 2a). This study could only demonstrate an overall increase in cellular metabolism in response to T_3 as indicated by the large increase in LDH activity, as well as rapid shifts in media pH in comparison to other hormonal treatments (unpublished observation). The inability of T_3 to affect preadipocyte differentiation is in contrast to mammalian species where thyroid hormones are considered an important regulator of preadipocyte growth and differentiation during the prenatal period (Martin et al., 1998). These data provide further evidence that the regulatory events in avian preadipocyte differentiation differ from mammalian preadipocyte differentiation.

IGF-I is a potent paracrine/autocrine adipogenic hormone in the mammal (Ramsay et al., 1989a; Butterwith, 1997). The chicken preadipocyte expresses IGF-I mRNA (Burt et al., 1992); however, it is not known if the chicken preadipocyte can secrete a functional protein. Recombinant human IGF-I has been shown to stimulate avian satellite cell proliferation (Pesall et al., 2001), suggesting that this recombinant peptide can bind to the avian receptor. The present study did not examine the potential actions of IGF-I on proliferation, but Butterwith (1997) clearly demonstrated that IGF-I can stimulate avian preadipocyte replication. Systemic infusion of recombinant human IGF-I in the chicken results in a reduction in size of the abdominal fat pad, but this may be due to partitioning of nutrients to other tissues. The present study could not demonstrate an adipogenic effect of 100 ng IGF-I/ml medium. Unlike any of the mammalian cell culture systems, IGF-I does not induce preadipocyte differentiation in this avian culture system. This may be due to the secretion of at least five or six IGF-binding proteins by chicken preadipocytes (Butterwith, 1994). Some of these binding proteins may inhibit interaction of IGF-I with its receptor (Baxter, 2000). Further studies will use IGF-I analogues that have limited affinity for the binding proteins to determine whether IGF-I is adipogenic for chicken preadipocytes.

Exogenous corticosterone administration promotes abdominal fat accretion in lean or fat chickens (Saadoun et al., 1987). Glucocorticoids have been used in vitro to promote preadipocyte differentiation of pig and rat preadipocytes, as well as 3T3-L1 cells (for review see Gregoire et al., 1998). The present study demonstrates that dexamethasone can promote preadipocyte differentiation as estimated by changes in the activity of enzyme markers for differentiation, although to a limited extent. However, the combination of insulin and dexamethasone hormones was most effective with

a low concentration of chicken serum. Presence of a high concentration of serum limited the response to the extent of dexamethasone alone. These data suggest that factors present in chicken serum are interfering with hormone action upon preadipocyte differentiation.

Irrespective of this, the combination of low serum, 100 nM insulin and 1 μM dexamethasone can replace high concentration of chicken serum in media for assessing markers of differentiation. However, these cells are still not accumulating significant quantities of lipid. The major site of lipogenesis in the bird is the liver. Enzymes associated with lipogenesis have relatively low activity in avian adipose tissue (Hermier, 1997). The majority of lipid is accumulated through the action of lipoprotein lipase on VLDL. Heparin reduces lipoprotein lipase degradation and thereby increases the secretion of lipoprotein lipase where it can function to promote fatty acid accumulation by adipocytes (Cupp et al., 1987). The present study used heparin to recruit lipoprotein lipase to enhance lipid accretion within the differentiated cells exposed to 2.5% chicken serum. The quantified histochemical data demonstrate that the combination of insulin, heparin and dexamethasone and 2.5% chicken serum stimulate the formation of fat cells as effectively as 10% chicken serum. Use of this culture technique may permit more effective assessment of the role of individual hormones or growth factors in the regulation of avian adipogenesis.

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